DISSERTATION ON

ROLE OF PLEURAL BIOPSY IN THE EVALUATION OF UNDIAGNOSED EXUDATIVE PLEURAL EFFUSION

submitted to The Tamil Nadu Dr.M.G.R.Medical University in partial fulfilment of the regulations for the award of the degree of

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THE TAMIL NADU Dr. M.G.R.MEDICAL UNIVERSITY
CHENNAI - 600 032

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BONAFIDE CERTIFICATE

Certified that this dissertation is the bonafide work of Dr.K.BHARATHI BABU on "**ROLE OF PLEURAL BIOPSY IN THE EVALUATION OF UNDIAGNOSED EXUDATIVE PLEURAL EFFUSION"** during his MD (Tuberculosis and respiratory disease) course from April 2008 to April 2011 at the INSTITUTE OF THORACIC MEDICINE AND GOVERNMENT GENERAL HOSPITAL – MADRAS MEDICAL COLLEGE, CHENNAI.

Prof.Dr.N.MEENAKSHI, M.D(TB&RD)., D.T.C.D.,
Director and Head of the Department,
Institute of Thoracic Medicine and Government General Hospital,
Chennai.

Prof.Dr.J.MOHANASUNDARAM, M.D., D.N.B., Ph.D.,
DEAN,
Madras Medical College and Government General Hospital,
Chennai – 600 003.

Madras Medical College & Government General Hospital

Chennai- 600003

DECLARATION BY THE SCHOLAR

I Hereby declare that the dissertation entitled "**ROLE OF PLEURAL BIOPSY IN THE EVALUATION OF UNDIAGNOSED EXUDATIVE PLEURAL EFFUSION**" submitted for the Degree of Doctor of Medicine in M.D, DEGREE EXAMINATION Branch XVIII TUBERCULOSIS & RESPIRATORY DISEASES is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship, fellowship or similar other titles. It had not been submitted to any other university or institution for the award of any degree or diploma.

Place: Chennai Signature of the scholar

Date : Name : **Dr.K.BHARATHI BABU**

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In keeping with the maxim, "All is well that ends well",I was able to carry out my study to my fullest satisfaction . I thank the guidance, encouragement, motivation and constant supervision extended to me by my respected **Director Prof.Dr.N.Meenakshi**.

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CONTENTS

INTRODUCTION

Pleural effusion is a collection of fluid in the Pleural space .It is not a disease but rather a complication of an underlying illness. Effusion can occur for a variety of reasons. Common classification systems divide pleural effusions into two categories of (1) Transudative pleural effusions and(2) Exudative pleural effusions.

Determining the cause of a pleural effusion is greatly facilitated by analysis of the pleural fluid. Thoracentesis is a simple bedside procedure that permits fluid to be rapidly sampled, visualized, examined microscopically, and quantified. A systematic approach to analysis of the fluid in conjunction with the clinical presentation should allow the clinician to diagnose the cause of an effusion.

- A definitive diagnosis is provided by the finding of malignant cells or specific organisms in the pleural fluid, can be established in approximately 25 percent of patients.

- Pleural effusion remains undiagnosed after routine tests in pleural fluid in many patients. so ,we need a simple and safe investigative tool to evaluate undiagnosed effusion .This study is designed to diagnose the cases of undiagnosed effusions by a simple and safe investigative tool.

Pleural fluid accumulates when pleural fluid formation exceeds pleural fluid absorption. Normally fluid enters the pleural space from the capillaries in the parietal pleura and is removed via the lymphatics situated in the parietal pleura. Fluid can also enter the pleural space from the interstitial spaces of the lung via the visceral pleura or from the peritoneal cavity via small holes in the diaphragm. The lymphatics have the capacity to absorb 20 times more fluid than is normally formed. Accordingly, a pleural effusion may develop when there is excess pleural fluid formation (from the interstitial spaces of the lung, the parietal pleura, or the peritoneal cavity) or when there is decreased fluid removal by the lymphatics.

When a patient is found to have a pleural effusion, an effort should be made to determine the cause . The first step is to determine whether the effusion is a transudate or an exudate. A transudative pleural effusion occurs when systemic factors that influence the formation and absorption of pleural fluid are altered. The leading causes of transudative pleural effusions are left ventricular failure and cirrhosis. An exudative pleural effusion occurs when local factors that influence

the formation and absorption of pleural fluid are altered. The leading causes of exudative pleural effusions are Tuberculosis, bacterial pneumonia, malignancy, viral infection, and pulmonary embolism. The primary reason to make this differentiation is that additional diagnostic procedures are indicated with exudative effusions to define the cause of the local disease.

AIMS AND OBJECTIVES

- To evaluate the role of closed Pleural biopsy in diagnosing exudative effusions not diagnosed by Pleural fluid analysis.

- To compare the yield of each test(Histopathological examination , Reverse Transcriptase Polymerase Chain Reaction and culture for Tuberculosis by BACTEC)in Pleural biopsy specimens of undiagnosed exudative Effusions .

REVIEW OF LITERATURE

INTRODUCTION

Pleural biopsy is helpful to reach an aetiological diagnosis in exudative pleural effusion, particularly when Malignancy is suspected or when the results of detailed pleural fluid study are inconclusive especially in a setup where Thoracoscopy is not available.

COMMON CAUSES OF PLEURAL EFFUSION

TRANSUDATIVE PLEURAL EFFUSIONS

Congestive heart failure

Cirrhosis

Nephrotic syndrome

Superior vena caval obstruction

Fontan procedure

Urinothorax

Peritoneal dialysis

Glomerulonephritis

Myxedema

Cerebrospinal fluid leaks to pleura

Hypoalbuminemia

Sarcoidosis

EXUDATIVE PLEURAL EFFUSIONS

Neoplastic diseases

Metastatic disease

Mesothelioma

Pyothorax-associated lymphoma

Bacterial infections

Tuberculosis

Fungal infections

Parasitic infections

Viral infections

Pulmonary embolization

Gastrointestinal disease

Heart diseases

Obstetric and gynecologic disease

Collagen vascular diseases

Drug-induced pleural disease

Asbestos exposure

Sarcoidosis

Hemothorax

Chylothorax

NEEDLE BIOPSY OF THE PLEURA

ABRAM'S NEEDLE

The Abram's needle consists of three parts: a large outer trocar, an inner cutting cannula, and an inner solid stylet. The end of the outer trocar is blunt so that the instrument will not lacerate the lung, but the bluntness of the instrument requires one to make a small scalpel incision in the anesthetized skin and subcutaneous tissue to permit insertion of the biopsy needle without undue force. This incision should be made along the lines of cleavage to minimize postoperative scarring. The inner cutting cannula fits tightly in the outer trocar and can be locked in one of two positions: (a) a closed position, in which the inner cannula obstructs the notch on the outer trocar to make the needle airtight, and (b) an open position, in which the inner cannula is slightly withdrawn so that the notch on the outer trocar is not occluded. An indicator knob in the hexagonal grip of the larger outer trocar indicates the position of the notch in the distal end of the trocar.

INDICATIONS

A needle biopsy of the pleura is currently recommended when tuberculous pleuritis is suspected and the pleural fluid ADA or interferon-gamma levels are not definitive. A needle biopsy of the pleura is also recommended when malignancy is suspected but the pleural fluid cytology is negative and thoracoscopy is not readily available.

With a needle biopsy of the pleura, a small piece of the parietal pleura is obtained for microscopic or microbiologic evaluation. The main diagnoses established with a needle biopsy of the pleura are tuberculous pleuritis and malignancy of the pleura. Currently, needle biopsy of the pleura is used less than in the past because the diagnosis of tuberculous pleuritis can be made by measuring the adenosine deaminase (ADA) or interferon-gamma level in the pleural fluid, and the diagnosis of pleural malignancy is usually established by pleural fluid cytology or thoracoscopy(1) .

CONTRAINDICATIONS

The main contraindication to a pleural biopsy is a bleeding diathesis.

Another contraindication to needle biopsies is the presence of an empyema(2). Other contraindications include an uncooperative patient and local cutaneous lesions such as pyoderma or herpes zoster infection.

A pleural biopsy should not be performed in patients who are taking anticoagulants or whose bleeding parameters are prolonged. If the platelet count is below 50,000/mm3, platelet transfusion should be given before the procedure is attempted.

If the patient has borderline respiratory failure, one should hesitate to perform a pleural biopsy because the production of a pneumothorax could precipitate respiratory failure.

TECHNIQUE

When there is a moderate or larger pleural effusion, the biopsy is usually done without image guidance. If the effusion is small or loculated, then either ultrasound or computed tomography (CT) can accurately identify the location of the fluid. Ultrasound is the preferred technique for guiding biopsy because it offers the advantage of a real-time approach to the biopsy and has the added advantages of the absence of ionizing radiation, portability, ready availability, and low expense. Because the patient can be imaged in the erect position, the depth of the fluid is maximized, thereby minimizing complications(3) .

The patient is positioned, and the site is selected as for diagnostic thoracentesis . The skin is cleaned, and the local anesthetic is administered as for diagnostic thoracentesis. Liberal amounts of lidocaine should be injected once the rib is passed to ensure adequate anesthesia of the parietal pleura. In general, if no fluid is obtained with the local anesthetic, biopsy should not be attempted. When pleural fluid has been obtained with the lidocaine syringe and needle, a pleural biopsy can be performed with an Abram's needle. A biopsy is sometimes attempted without free pleural fluid. If there is no fluid, the procedure should be performed with ultrasonic or CT guidance .

COMPLICATIONS

Pleural biopsy has the same complications as diagnostic thoracentesis. One might expect pneumothorax to be more common with pleural biopsy than with thoracentesis for two reasons. First, the atmosphere has much more opportunity to be in communication with the pleural space with the biopsy (particularly when the Cope needle is used). Second, when the biopsy specimen is obtained, the visceral pleura may be inadvertently incised, leaving a small bronchopleural fistula that can lead to a large pneumothorax. However, the incidence of pneumothorax and the requirement for tube thoracostomy are comparable after thoracentesis and pleural biopsy (4). This is probably because more experienced individuals usually perform the pleural biopsy.

The second major complication of pleural biopsy is bleeding. If an intercostal artery or vein is inadvertently biopsied, a hemothorax can result (5,6). There is one case report of an arteriovenous fistula from an intercostal artery to an intercostal vein developing after pleural biopsy (7).

TUBERCULOUS PLEURAL EFFUSION

When a tuberculous pleural effusion occurs in the absence of radiologically apparent TB, it may be the sequel to a primary infection 6 to 12 weeks previously or it may represent reactivation TB (8). In industrialized countries, more pleural effusions may be due to reactivation than are due to post primary infection (8). However, in a recent study from San Francisco, pleural TB cases were approximately two times more likely to be clustered (as assessed by genotyping of the mycobacterial organisms) than were pulmonary TB and three times more likely to be clustered than nonrespiratory TB cases(9).

The tuberculous pleural effusion is thought to result from rupture of a subpleural caseous focus in the lung into the pleural space(10). Supporting evidence comes from the operative findings of Stead et al.(11) , who reported that they could demonstrate a caseous tuberculous focus in the lung contiguous with the diseased pleura in 12 of 15 patients with tuberculous pleuritis. The remaining three patients in this series were found to have parenchymal TB, although these patients did not have caseous foci adjacent to the pleura.

It appears that delayed hypersensitivity plays a large role in the pathogenesis of tuberculous pleural effusion. The hypersensitivity reaction is initiated when tuberculous protein gains access to the pleural space.

It is probable that delayed hypersensitivity also plays a large role in the development of tuberculous pleural effusions in humans. The mycobacterial cultures of the pleural fluid from most patients with tuberculous pleural effusions are negative(12,13).

Although delayed hypersensitivity to tuberculous protein is probably responsible for most clinical manifestations of tuberculous pleuritis, many patients when first evaluated have a negative PPD skin test. The explanation for this paradox may be a combination of two factors. First, in some(14), but not in all (15) patients with tuberculous pleuritis, a circulating mononuclear adherent cell suppresses the specifically sensitized circulating T lymphocytes in the peripheral blood. Second, there may be sequestration of PPD-reactive T lymphocytes in the pleural space involving both Leu-2 (suppressor/cytotoxic) and Leu-3 (helper) positive T cells(15).

REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

PCR is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of a particular DNA sequence.

HISTORY:

A 1971 paper in the journal of molecular biology of Kleppe and co-workers first described a method using an enzymatic assay to replicate a short DNA template with primers in vitro. Invention of the PCR in 1983 is generally credited to Karry mullis. The discovery of taq polymerase in 1976 (DNA polymerase purified from thermophilic bacterium thermos aquaticus) paved the way for dramatic improvement of the PCR technique. Karry mullis was awarded the Nobel Prize in chemistry in 1993.

RTPCR is an improvement from the conventional PCR and can detect the m-RNA of the organism under study.

- RTPCR targeting 85 B gene is very specific for Mycobacterium tuberculosis, so that it is negative for Non-Tuberculous Mycobacterium.
- m-RNA detected by RTPCR has a half life for only few minutes and can detect only viable Mycobacterium .

BACTEC CULTURE

The rapid radiometric culture system or BACTEC (Becton-Dickinson) has been accepted for the culture isolation of mycobacteria using an enriched Middlebrook 7H12 containing 14C labeled palmitic acid. This medium is otherwise called BACTEC 12B. Mycobacterial growth is determined by the utilization of 14C with release of 14CO2 by the multiplying mycobacteria and is detected in an ionic chamber with electronic detector in the BACTEC instrument. In comparison to the conventional M. tuberculosis culture using Lowenstein-Jensen Media, the BACTEC system gives early culture results with differentiation of M. tuberculosis from mycobacteria other than M. tuberculosis(16,17,18,19).

MALIGNANT PLEURAL EFFUSION

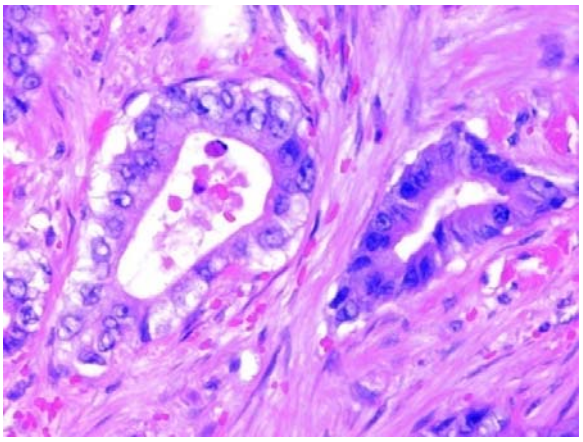
Carcinomas of the lung and breast and lymphomas account for approximately 75% of malignant pleural effusions . lung cancer is the leading cause of malignant pleural effusion (20). When patients with lung cancer are first evaluated, approximately 15% have a pleural effusion (21).

Needle biopsy of the pleura can establish the diagnosis of a malignant pleural effusion. The percentage of positive pleural biopsies in patients with malignant pleural disease ranges from 39% to 75% (22,23,24). In general, pleural fluid cytology is superior to pleural biopsy in establishing the diagnosis of pleural malignancy. The diagnosis of malignancy was made in 13 of 15 patients (87%) with the CT-guided biopsy but in only 8 of 17 patients (47%) with the Abram's needle (25).

ADENOCARCINOMA

Adenocarcinoma of the lung is a form of non-small cell lung cancer. Eighty percent of lung cancers are non-small cell cancers, and of these, about 50% are adenocarcinomas. Adenocarcinoma of the lung begins in the outer parts of the lung, and it can be present for a long time before it is diagnosed. It is the type of lung cancer most commonly seen in women and is often seen in non-smokers.

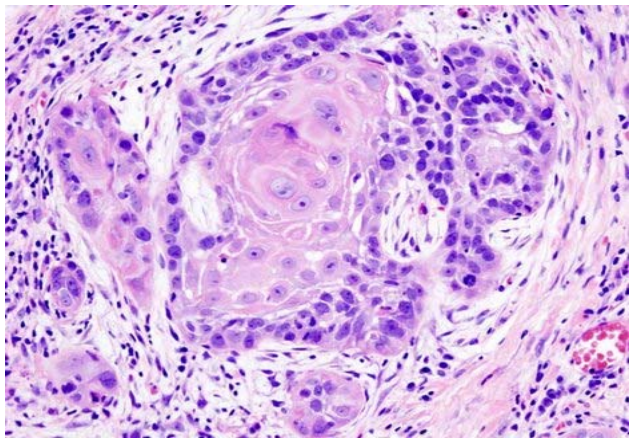
The most common type of lung cancer in lifelong non-smokers is the adenocarcinoma.This cancer usually is seen peripherally in the lungs, as opposed to small cell lung cancer and squamous cell lung cancer, which both tend to be more centrally located. The adenocarcinoma has an increased incidence in smokers, but is also the most common type of lung cancer seen in non-smokers.



Adenocarcinoma of the lung tends to stain mucin positive as it is derived from the mucus producing glands of the lungs. Similar to other adenocarcinoma, if this tumor is well differentiated (low grade) it will resemble the normal glandular structure. Poorly differentiated adenocarcinoma will not resemble the normal glands (high grade) and will be detected by seeing that they stain positive for mucin (which the glands produce).

SQUAMOUS CELL CARCINOMA

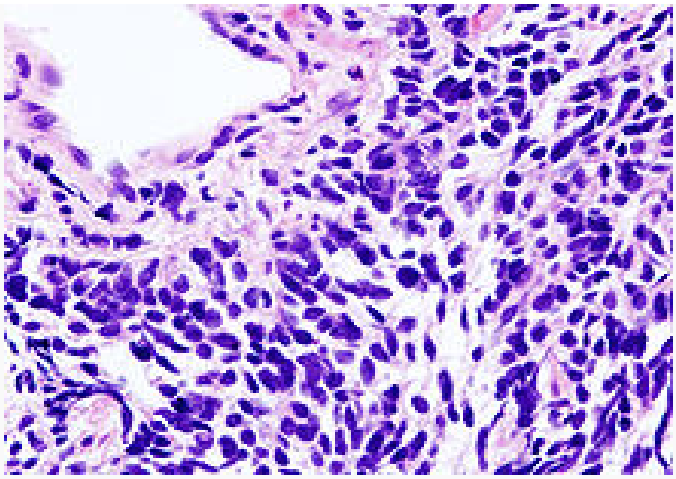
Squamous cell lung cancer is a form of non-small cell lung cancer. Squamous cell lung cancers usually begin in the bronchial tubes (large airways) in the central part of the lungs.



Squamous Cell Carcinoma cells are large, flattened and stratified with a high cytoplasm to nucleus ratio. Key diagnostic features include the presence of intra cytoplasmic keratin which may be linked to the presence of intercellular bridges and squamous pearl formation. Most Squamous Cell Carcinomas arise centrally within the main, lobar, segmental or subsegmental bronchi but some occur more peripherally. The tumour mass generally extends into the lumen of the airway with invasion into the underlying wall.

SMALL CELL LUNG CARCINOMA

Small cell lung cancer (SCLC) is a tumor of extremes. Untreated, it is one of the most highly virulent malignancies known, with a life expectancy best measured in days to weeks. On the other hand, it displays exquisite chemosensitivity, resulting in partial or complete responses in the vast majority of cases. Unfortunately, although many patients can be rendered free of clinical evidence of disease, most quickly relapse and die from this malignancy.



Like all other lung cancers, SCLC is linked to a variety of environmental risk factors. By far the strongest association is with the use of tobacco: Up to 98 percent of SCLC patients have a history of smoking. Occupational risks for SCLC include exposure to

bischloromethyl ethers, nickel, vinyl chloride, asbestos, cadmium, and radon daughters (in uranium miners). Other types of radiation exposure also appear to be significant risk factors, with an increased incidence of SCLC in atomic bomb survivors and patients (typically those with breast cancer or Hodgkin's lymphoma) exposed to therapeutic irradiation.

OTHER RELATED STUDIES

The study done by Biswajit Chakrabarti, MBBS, FRCP; Ida Ryland, MSc;John Sheard, MD, FRCPath; Christopher J. Warburton, MD, FRCP; and John E. Earis, MD, FRCP on "**The Role of Abrams Percutaneous Pleural Biopsy in the Investigation of Exudative Pleural Effusions"** says blind Abrams needle biopsy is being superseded by image-guided biopsies and thoracoscopy, both of which have been demonstrated to have a higher diagnostic yield in malignant disease.(26,27). On conclusion of this study (minimum of 1 year after biopsy), malignancy was confirmed in 46 cases (61%), of which 21 cases (46%) were diagnosed by Abrams pleural biopsies. In the 75 patients studied, the overall diagnostic sensitivity of initial blind biopsy was 38% (rising to 43% when pleural tissue was obtained), with a

negative predictive value of 40%. If those Abrams biopsies that failed to obtain pleural tissue were excluded from the study, the sensitivity for diagnosing malignancy increased to 51%.**(Chest 2006;129;1549-1555 DOI 10.1378/chest.129.6.1549).**

The study done by Nadia A. Hasaneen, MD, PhD; Maysaa E. Zaki, MD, PhD; Hala M. Shalaby, MD; and Ahmad S. El-Morsi, MD, PhD on "**Polymerase Chain Reaction of Pleural Biopsy Is a Rapid and Sensitive Method for the Diagnosis of Tuberculous Pleural Effusion"** had the overall accuracy of PCR of pleural biopsy was similar to the results of pleural biopsy culture, however, PCR of the pleural biopsy was much faster in reaching diagnosis. PCR of pleural biopsy is a useful method when used in combination with the BACTEC culture system and histopathologic examination of pleural biopsy to reach a rapid diagnosis of tuberculous pleural effusion. **(CHEST 2003; 124:2105–2111 DOI 10.1378/chest.124.6.2105)**

The study done by Renda Soylemez Wiener, MD; Phyllis Della-Latta, PhD; and Neil W. Schluger, MD, FCCP on **"Effect of Nucleic Acid Amplification for Mycobacterium tuberculosis on Clinical Decision Making in Suspected Extrapulmonary Tuberculosis"** had the following results The NAAT proved to be a sensitive and specific

test for detection of *M tuberculosis* in extrapulmonary specimens but did not weigh heavily in clinical decision making at our hospital. Judicious use of these tests may improve the accuracy and speed of diagnosis of extrapulmonary tuberculosis, while helping to eliminate unnecessary antituberculous treatment in patients without Tuberculosis. **(CHEST 2005; 128:102–107 DOI 10.1378/chest.128.1.102)**

The study done by W R Salyer, J C Eggleston and Y S Erozan on **" Efficacy of Pleural Needle Biopsy and Pleural Fluid Cytopathology in the Diagnosis of Malignant Neoplasm Involving the Pleura"** has compared the efficacy of pleural needle biopsy and pleural fluid cytopathology in the diagnosis of pleural neoplasm Cytologic studies alone yielded a higher percentage of cancer diagnoses than did the biopsies alone. A diagnosis was established in 90 percent of the patients with pleural tumor when both studies were performed. **(*Chest* 1975;67;536-539 DOI 10.1378/chest.67.5.536)**

The study conducted by Sudipta Pandit, Arunabha Datta Chaudhuri, Sourin Bhuniya Saikat Datta, Atin Dey, Pulakesh Bhanja at the Department of Chest Medicine, R.G. Kar Medical College, Kolkata, India on **"Role of pleural biopsy in etiological diagnosis of pleural effusion"** says Malignancy was the most common etiology, followed by

tuberculosis. Pleural biopsy was done in 72 patients. Pleural tissue was obtained in 62 cases. Malignancy was diagnosed in 24, tuberculosis in 20 and non-specific inflammation in 18, on histopathological examination. Out of 20 histological proven tuberculosis cases adenosine de-aminase (ADA) was more than 70 u/l in 11 cases and concludes that malignancy is more common than tuberculosis, particularly in elderly. When thoracoscope is not available, pleural fluid cytology and pleural biopsy can give definite diagnosis. Pleural fluid ADA ≥ 70 u/l is almost diagnostic of tuberculosis, where pleural biopsy is not recommended.(**Lung India, Year 2010, Volume 27, Issue 4 [p. 202-204] DOI: 10.4103/0970-2113.71941**)

MATERIALS AND METHODS

STUDY DESIGN

This is a Prospective (Observational) study designed to evaluate the role of closed pleural biopsy in undiagnosed exudative Pleural effusion .

STUDY CENTER

The study was done at the Department of Thoracic Medicine – Government General Hospital, Chennai.

STUDY DURATION

January 2010 to June 2010

STUDY POPULATION

Patients with exudative pleural effusion remain undiagnosed after pleural fluid analysis.

Proforma was designed and ethical clearance was obtained. A written informed consent was obtained from all the patients included in the study after explaining in detail the nature and purpose of the study.

INCLUSION CRITERIA

- Exudative effusion which is negative for Malignant cells, Acid Fast Bacillus, Gram stain and Non Tuberculous culture in Pleural fluid .

EXCLUSION CRITERIA

- Exudative effusion positive for Malignant cells
- Exudative effusion positive for Tuberculosis in Pleural fluid smear
- Exudative effusion positive for Gram stain or Non-Tuberculous culture
- Parenchymal lesion in the X-ray suggestive of Tuberculosis or Malignancy or parapneumonic effusion
- Uncooperative patients
- Coagulation disorders
- Dry pleural tapping
- Empyema
- Uremia

STUDY PROCEDURE

Patients with undiagnosed exudative pleural effusion, fulfilling Inclusion criteria were admitted in Thoracic Medicine ward at Government General Hospital.

Informed & Written consent obtained from the patient .

Investigations were done to rule out Renal diseases and Coagulation abnormality. Pleural fluid sent for basic biochemical and microbiological investigations.

Pleural biopsy was performed using ABRAM'S pleural biopsy needle under strict aseptic precaution under local anaesthesia and the specimen was sent for the following investigations

- Tissue – Histopathological Examination
- Tissue – Reverse Transcriptase Polymerase Chain Reaction for Tuberculosis.
- Tissue – Culture for Tuberculosis by BACTEC

METHOD OF PLEURAL BIOPSY

The patient was positioned, and the site was selected either clinically or by image guidance. The skin was cleaned, and the local anesthetic was administered as for diagnostic thoracentesis. Liberal amounts of lignocaine was injected once the rib is passed to ensure adequate anesthesia of the parietal pleura.

Skin incision was made at the selected site to insert the Abram's pleural biopsy needle, the stylet was placed in the inner cannula, which, in turn, was placed in the outer trocar. The inner cannula was twisted clockwise to close the distal notch of the outer trocar. The needle was pushed into the pleural space by exerting firm pressure on the stylet. Because the needle has a large diameter and is blunt, a substantial amount of pressure was needed. Usually, a pop was heard as the needle enters the pleural space. The inability to pass the needle into the pleural space is usually because of an insufficiently large skin incision. At times, the ribs were too close together to allow the needle to pass. In such situations, rotation of the patient's arm and shoulder over his or her head frequently separated the ribs sufficiently.

Once the tip of the needle is thought to be in the pleural space, the inner stylet was removed, and with the inner cannula in the closed position, a syringe was attached to the connection on the inner cannula. Then, the inner cannula was rotated counter-clockwise in the outer trocar so that the distal notch was locked open. At this time, pleural fluid was aspirated for diagnostic studies. When the desired fluid has been obtained, the inner cannula of the needle was rotated clockwise to occlude the distal notch so that the syringe can b changed without creating a pneumothorax. A 10- to 20-mL syringe was then attached to the needle, and the inner cannula was rotated to open the distal notch. The entire needle was then rotated so that the knob on the outer trocar was inferior. This was important so that the blood vessels and nerves that lie immediately below the rib were not biopsied. The biopsy needle was then slowly withdrawn with constant aspiration until it hooked onto the pleura . When the needle hooks, one can be sure that parietal pleura was in the notch of the needle if pleural fluid can still be aspirated through the syringe. When the needle was hooked on the pleura, the outer trocar was held firmly with one hand while the inner cannula was rotated into the closed position with the other hand to cut off a small piece of parietal pleura . Usually, mild resistance was met immediately

before the needle was completely closed, and this resistance is because the inner cannula severing the entrapped pleura for the biopsy specimen.

Once the initial biopsy specimen was obtained, the needle can either be withdrawn from the pleural space in the closed position, the pleural biopsy specimen was found in the tip of the needle. Reinsertions of the needle are through the same tract, however, and are easier than the original insertion. The biopsy procedure can be repeated without removing the biopsy needle. Whenever the Abram's pleural biopsy needle was withdrawn from the pleural space, the biopsy tract was occluded with a finger immediately after the needle was withdrawn to decrease the likelihood of a pneumothorax.

At least six separate biopsy specimens were obtained. Four were placed in formalin and were taken to the pathology laboratory, and the other two were placed in a sterile container and sent to the laboratory for BACTEC culture and Reverse Transcriptase Polymerase Chain Reaction targeting 85B gene of Mycobacterium Tuberculosis.

Once the biopsy specimens were obtained, a therapeutic thoracentesis was performed through the Abram's needle. The pleural fluid should was removed only after obtaining the biopsy specimens

because the pleural fluid separates the parietal and visceral pleura, and increases the safety of the procedure.

When the Abram's needle was withdrawn for the last time, the biopsy site was massaged for a short time to eradicate the needle tract. Then a small adhesive bandage was placed over the biopsy incision in a crosswise manner to act as a butterfly-type dressing. Chest radiographs were obtained on all patients after pleural biopsies.

TREATMENT

Patients with conclusive evidence of Tuberculosis or Malignancy were treated with Anti Tuberculous Treatment in case of Tuberculosis and for the underlying Malignancy in case of Malignant disease . The patients left undiagnosed (11 out of the 51 cases) even after pleural biopsy were started on Anti Tuberculous Treatment and were further evaluated and followed up. Five out of eleven patients were found to have Malignancy of which two patients had a peripheral mass in the repeat CT chest taken after draining all the pleural fluid an CT guided bipsy of the mass revealed Malignancy, one patient was diagnosed by Thoracoscope outside our center, one was diagnosed after metastasis to cervical node in Fine Needle Aspiration Cytology of cervical node and the other patient was a case of male carcinoma breast operated 4 years back and completed chemotherapy and radiotherapy had a disseminated disease on further evaluation. The other six patients responded to Anti Tuberculous Treatment.

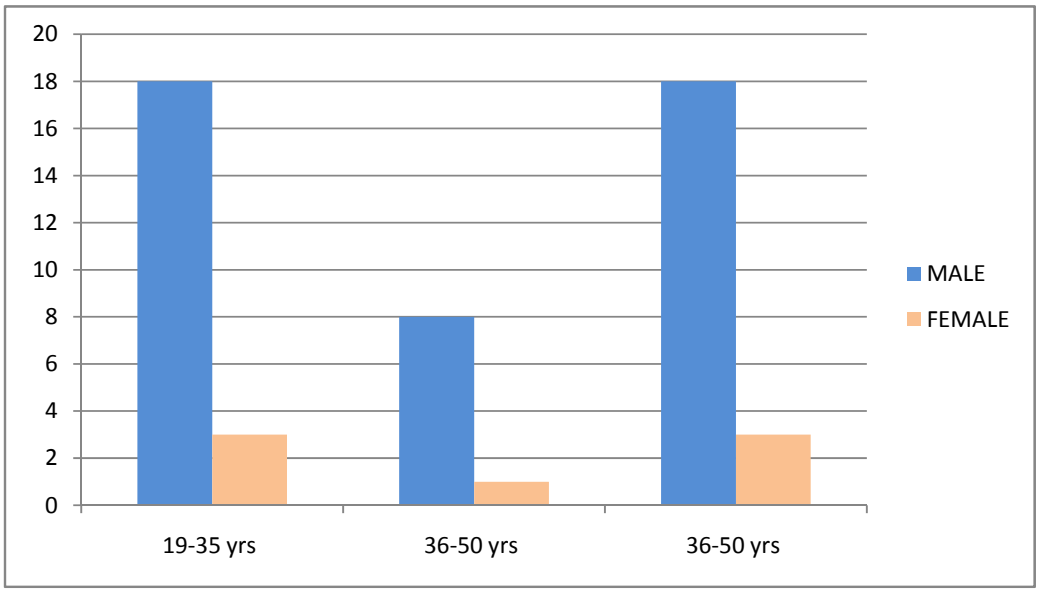
RESULTS

Pleural biopsy was done in 51 patients (44 Males and 7 Females) and the results of Histopathological Examination, BACTEC culture and Reverse Transcriptase Polymerase Chain Reaction on the pleural biopsy specimens were analysed and the results of which were as follows .

AGE & SEX DISTRIBUTION

YEARS	19-35 yrs	36-50 yrs	>50 yrs
MALE	18	8	18
FEMALE	3	1	3

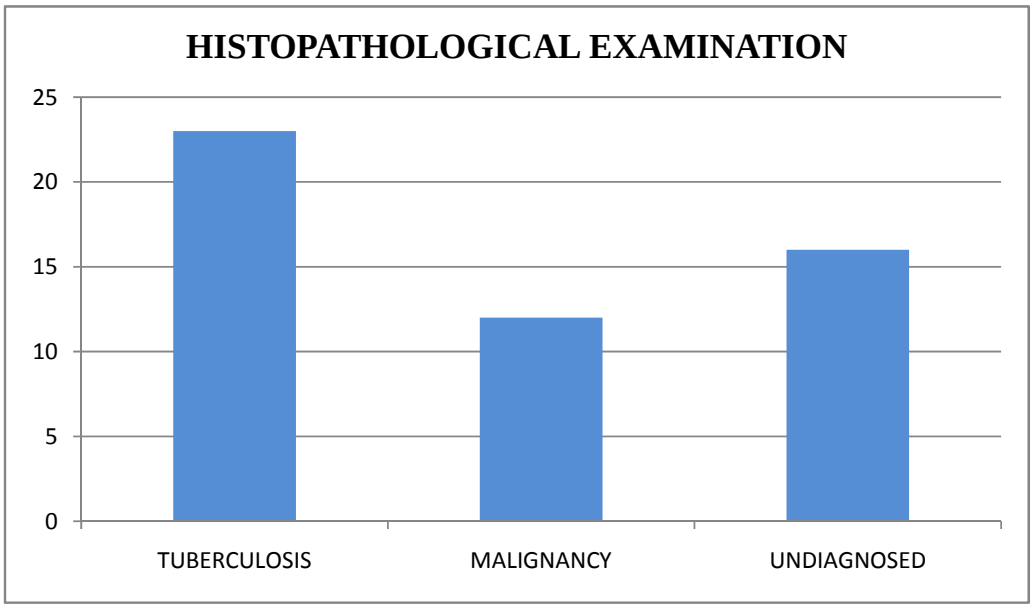
During the study period of 6 months, Pleural biopsy was done in 51 patients of undiagnosed exudative effusions out of which there were 7 females and 44 males. There is a clear male predominance with the incidence more in Younger age and Elder age and a relatively lower incidence in middle age group .



YIELD OF HPE IN PLEURAL BIOPSY

HPE	FREQUENCY (n=51)	PERCENT
TUBERCULOSIS	23	45.1%
MALIGNANCY	12	23.5%
UNDIAGNOSED	16	31.4%

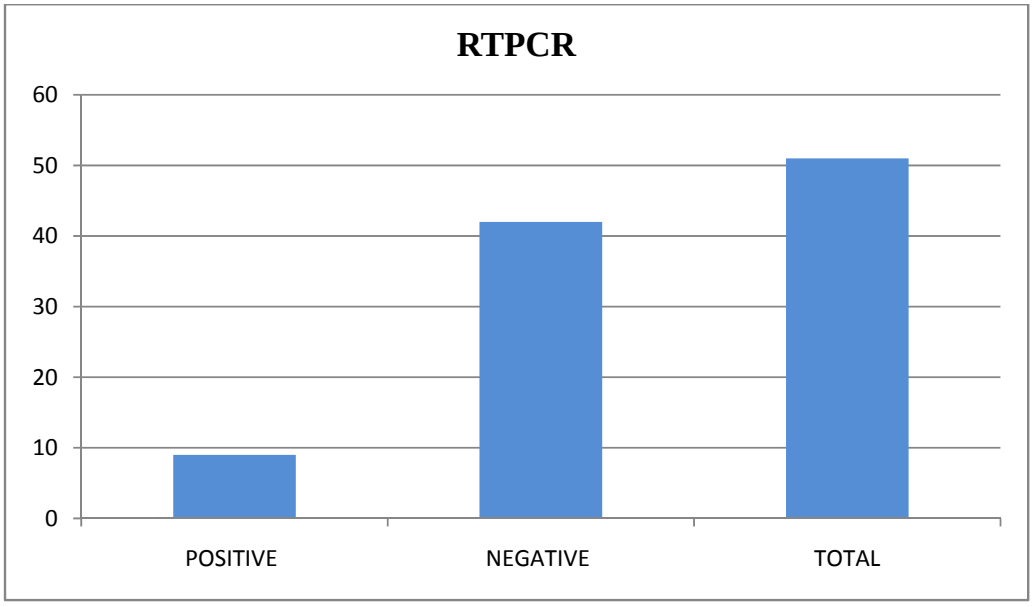
Histopathological Examination diagnosed 45.1% of biopsy specimens as Tuberculosis and 23.5% as Malignancy. The rest 31.4% of case were left undiagnosed after Histopathological Examination of Pleural biopsy.



RTPCR IN PLEURAL BIOPSY

RTPCR	FREQUENCY (n=51)	PERCENTAGE
POSITIVE	9	17.6%
NEGATIVE	42	82.4%

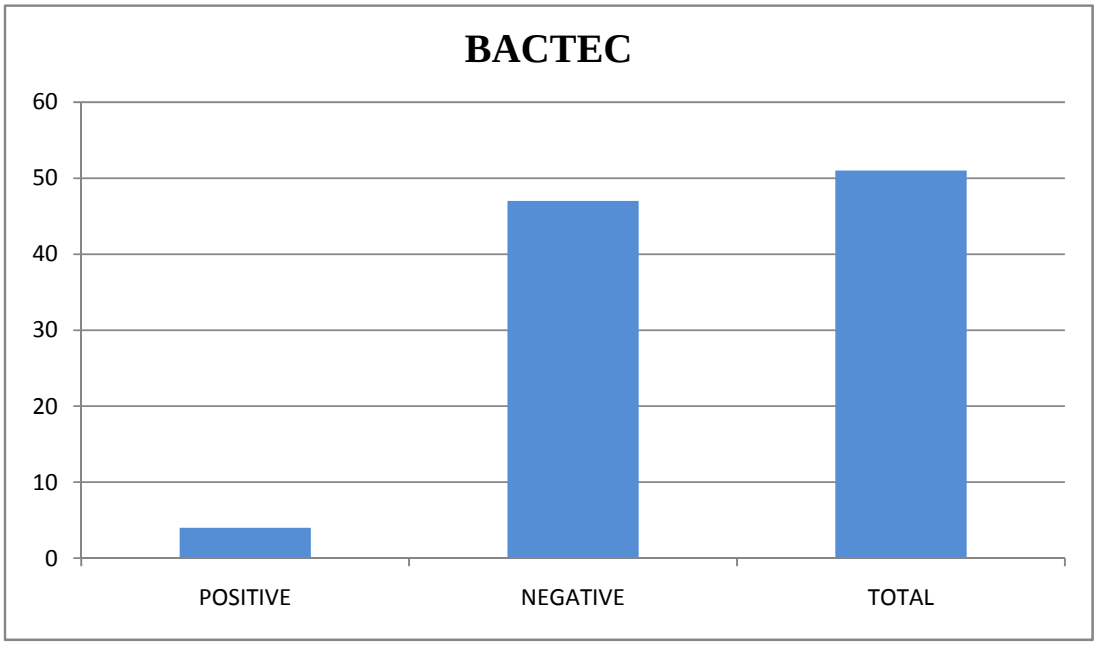
Reverse Transcriptase Polymerase Chain Reaction was positive in 17.6% of the cases and was negative in 82.4% of the cases (That is 9 out of the 51 cases were positive for RTPCR).



BACTEC IN PLEURAL BIOPSY

BACTEC	FREQUENCY (n=51)	PERCENTAGE
POSITIVE	4	7.84%
NEGATIVE	47	92.16%

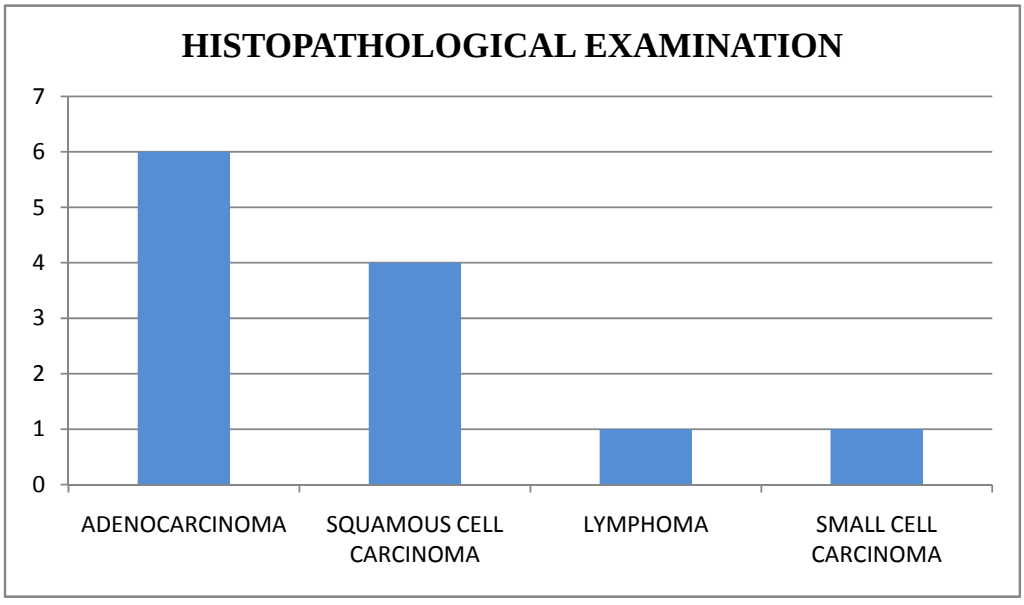
BACTEC was able to diagnose only 4 cases in the pleural biopsy specimens. BACTEC was positive in 7.84% of the cases and was negative in92.16% of the cases.



INCIDENCE OF PLEURAL MALIGNANCIES

HPE	FREQUENCY (n=12)	PERCENT
ADENOCARCINOMA	6	50%
SQUAMOUS CELL CARCINOMA	4	33.33%
LYMPHOMA	1	8.33%
SMALL CELL CARCINOMA	1	8.33%

Out of the malignancies proved in the study Adenocarcinoma topped the list with 50% followed by Squamous cell carcinoma with 33.33% and then by Lymphoma and Small cell carcinoma with 8.33% each.

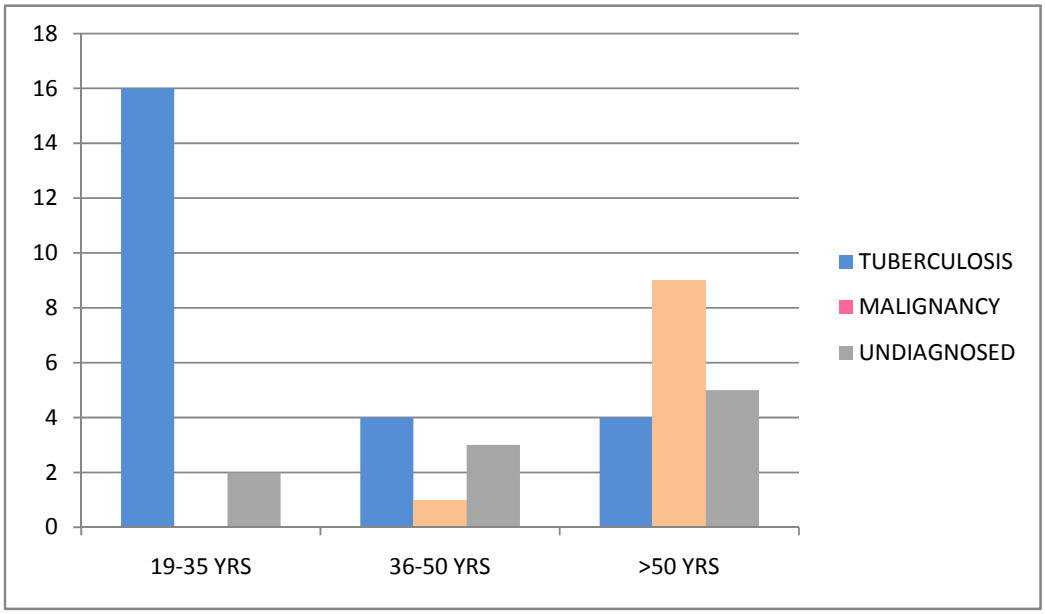


YIELD OF PLEURAL BIOPSY

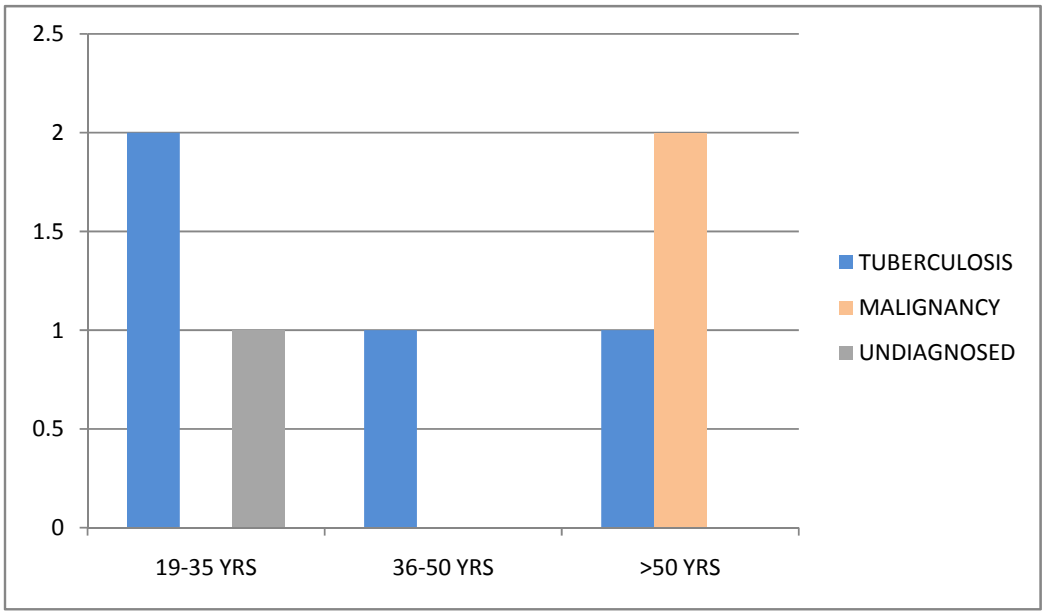
	RESULTS	**19-35 yrs**	**35-50 yrs**	**>50 yrs**
MALE	TUBERCULOSIS	16	4	4
	MALIGNANCY	0	1	9
	UNDIAGNOSED	2	3	5
FEMALE	TUBERCULOSIS	2	1	1
	MALIGNANCY	0	0	2
	UNDIAGNOSED	1	0	0
TOTAL		21	9	21

This clearly shows that Tuberculous Pleural effusion is more common in the Younger age group and Malignant Pleural effusion more common in the Elder age in both males and females.

MALE


18
16
14
12
10
8
6
4
2
0
19-35 YRS
36-50 YRS
>50 YRS
TUBERCULOSIS
MALIGNANCY
UNDIAGNOSED

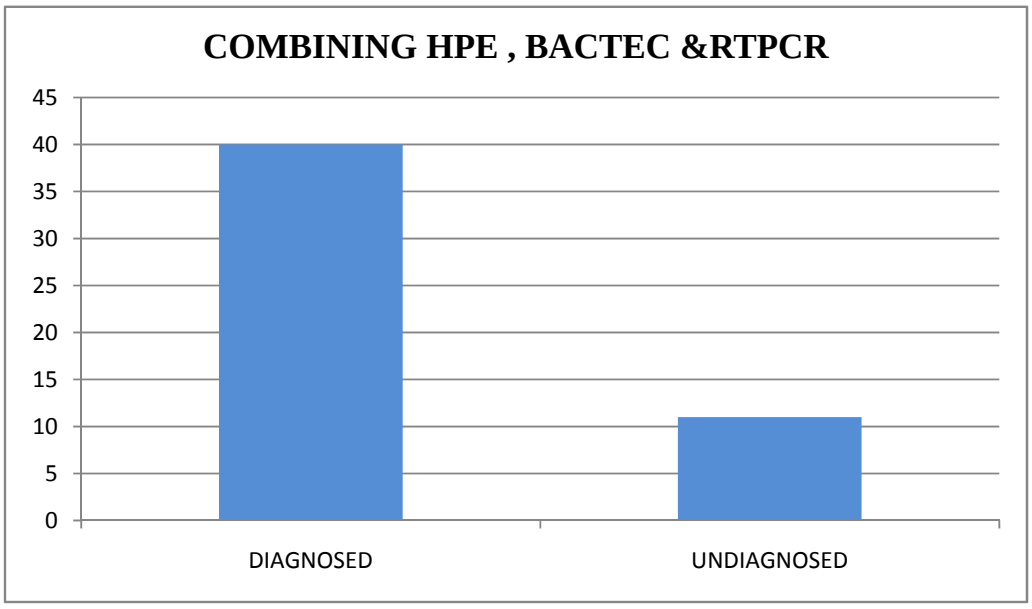
FEMALE


2.5
2
1.5
1
0.5
0
19-35 YRS
36-50 YRS
>50 YRS
TUBERCULOSIS
MALIGNANCY
UNDIAGNOSED

CASES DIAGNOSED BY PLEURAL BIOPSY

COMBINING HPE , BACTEC &RTPCR	FREQUENCY (n=51)	PERCENT %
DIAGNOSED	40	78.5%
UNDIAGNOSED	11	21.5%

About 78.5% of cases were diagnosed when combining Histopathological Examination, BACTEC culture and Reverse Transcriptase Polymerase Chain Reaction on Pleural biopsy specimen. 21.5% of cases were left undiagnosed even after combining all the three tests.



FOLLOW UP . . .

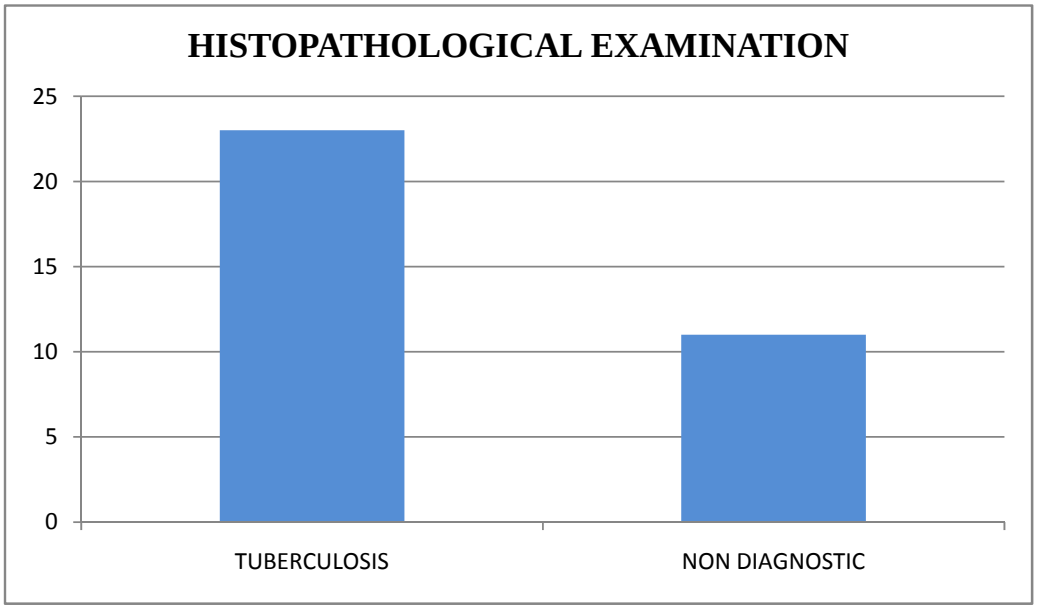
Five out of the eleven patients left undiagnosed after pleural biopsy were found to have Malignancies . The other six responded to Anti-Tuberculous Treatment.

The study population was divided into two groups of Tuberculous Pleural Effusion and Malignant Pleural Effusion based on the final diagnosis and the yield of each test in Tuberculous and Malignant effusion are discussed separately as follows.

YIELD OF HPE IN TUBERCULOUS EFFUSION

HPE	FREQUENCY (n=34)	PERCENT %
TUBERCULOSIS	23	67.6%
NEGATIVE	11	32.4%

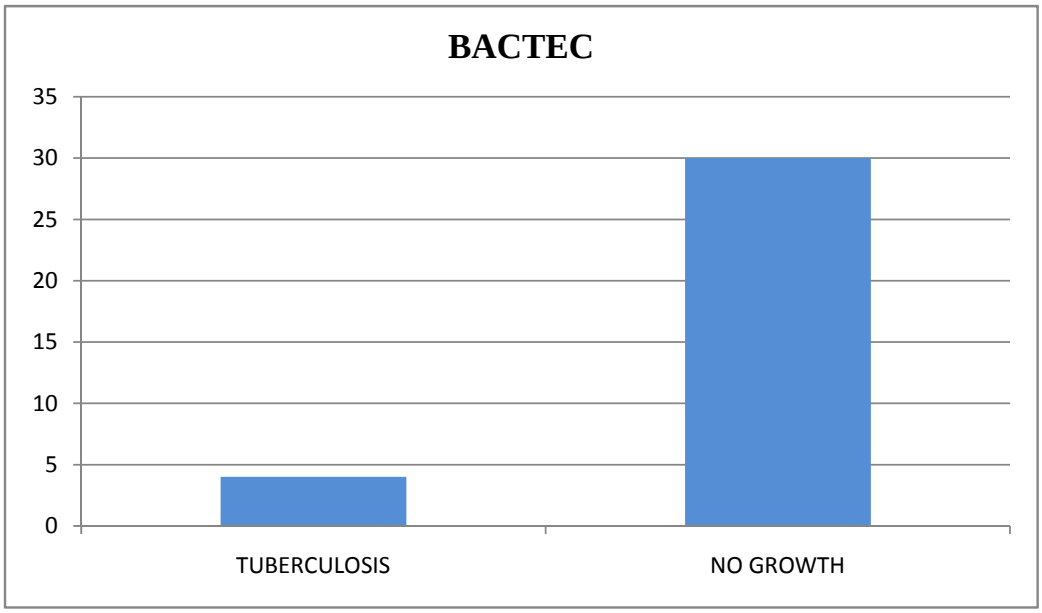
Histopathological Examination was able to diagnose 67.6% of Tuberculous cases and was Non-diagnostic in 32.4% of the Tuberculous cases.



YIELD OF BACTEC IN MYCOBACTERIAL INFECTION

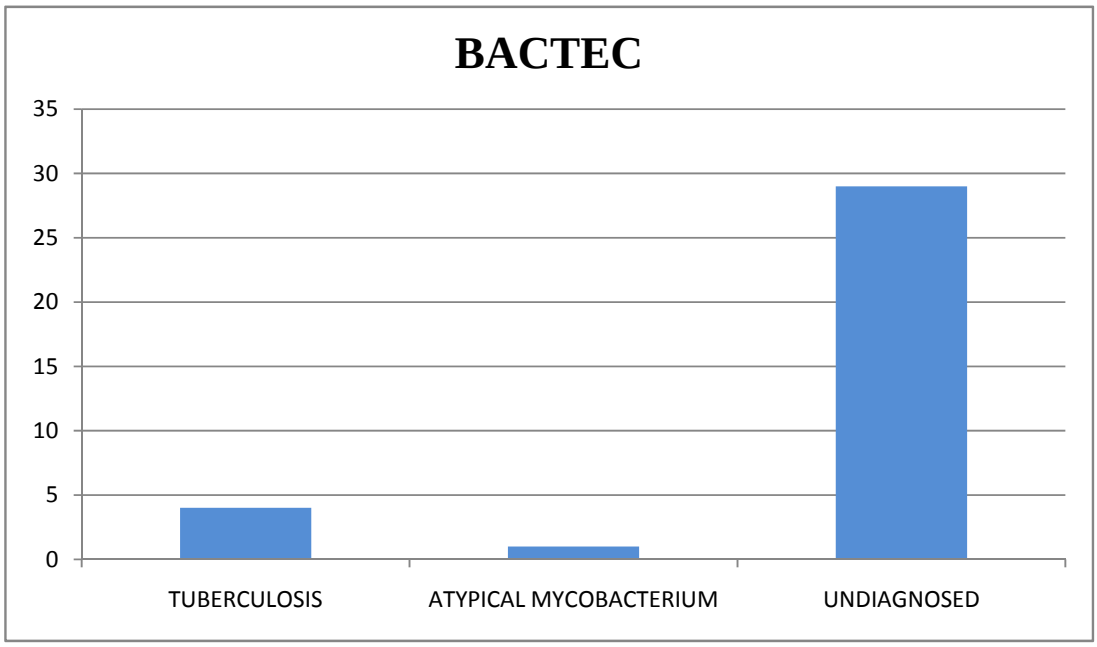
BACTEC	FREQUENCY (n=34)	PERCENT %
POSITIVE	4	11.8%
NEGATIVE	30	88.2%

BACTEC culture was able to diagnose only 11.8% of Tuberculous cases and was Non-diagnostic in 88.2% of the Tuberculous cases.



YIELD OF BACTEC IN MYCOBACTERIAL INFECTION

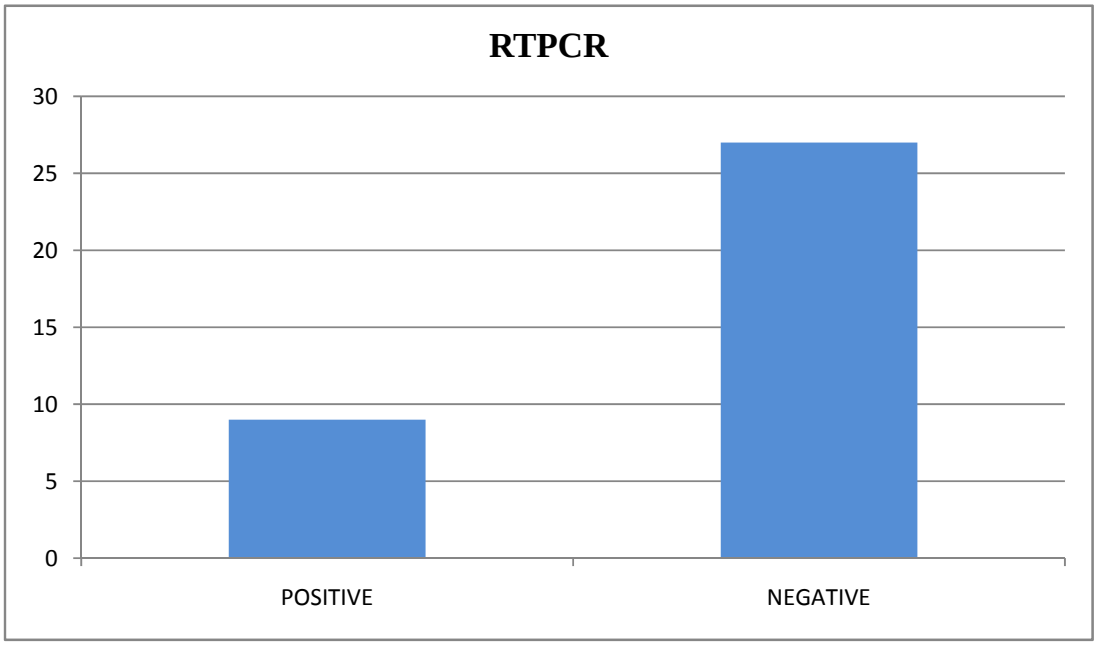
BACTEC was able to diagnose a case of Pleural effusion due to Non-Tuberculous Mycobacterium (Mycobacterium fortuitum). The patient started on Category-I Anti Tuberculous Treatment along with Macrolides. The patient improved well .



YIELD OF RTPCR IN TUBERCULOUS EFFUSION

RTPCR	FREQUENCY (n=34)	PERCENT
POSITIVE	9	26.1%
NEGATIVE	27	73.9%

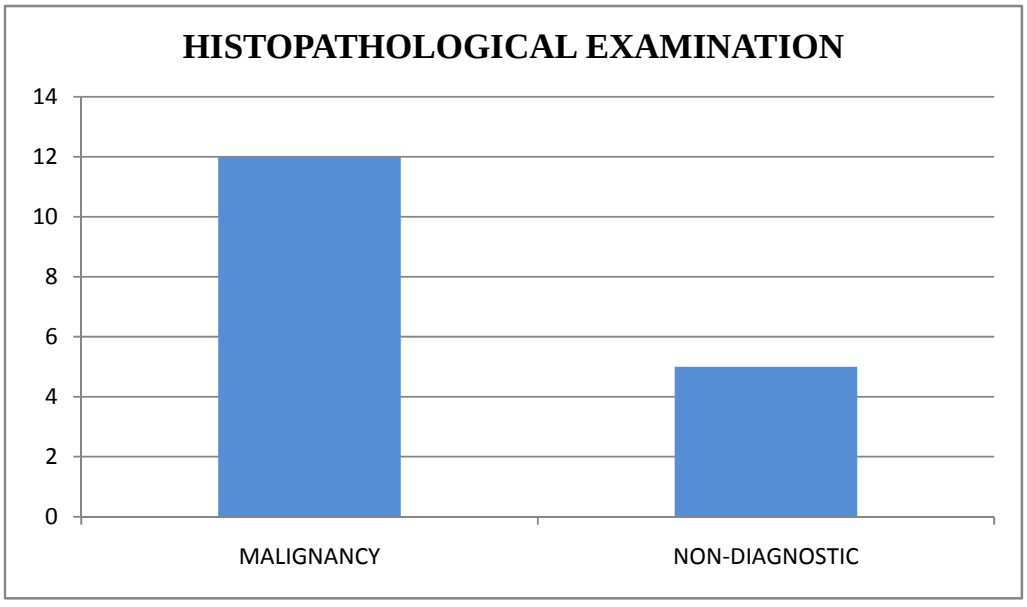
RTPCR was able to diagnose 26.1% of Tuberculous cases and was Non-diagnostic in 73.9% of the Tuberculous cases.



YIELD OF HPE IN MALIGNANT PLEURAL EFFUSION

HPE	FREQUENCY (n=17)	PERCENT %
DIAGNOSED	12	70.6%
NEGATIVE	5	29.4%

Histopathological Examination diagnosed 70.6% of malignant cases and only 29.4% needed further evaluation to prove their malignancy.



SENSITIVITY AND SPECIFICITY

HISTOPATHOLOGICAL EXAMINATION

HPE	TUBERCULOUS CASES	NON-TUBERCULOUS CASES
POSITIVE	23	0
NEGATIVE	11	17

HISTOPATHOLOGICAL EXAMINATION	PERCENTAGE
SENSITIVITY	67.64%
SPECIFICITY	100%
POSITIVE PREDICTIVE VALUE	100%
NEGATIVE PREDICTIVE VALUE	60.71%

There were no False positive results with Histopathological Examination making the Specificity and Positive predictive value as 100%. The Sensitivity was 67.64% and Negative predictive value was 60.71%.

SENSITIVITY AND SPECIFICITY

BACTEC

BACTEC	TUBERCULOUS CASES	NON-TUBERCULOUS CASES
POSITIVE	4	0
NEGATIVE	30	17

BACTEC	PERCENTAGE
SENSITIVITY	11.8%
SPECIFICITY	100%
POSITIVE PREDICTIVE VALUE	100%
NEGATIVE PREDICTIVE VALUE	36.17%

There were no False positive results with BACTEC culture making the Specificity and Positive predictive value as 100%. The Sensitivity was only 11.8% and Negative predictive value was 36.17%.

SENSITIVITY AND SPECIFICITY

RTPCR

RTPCR	TUBERCULOUS CASES	NON-TUBERCULOUS CASES
POSITIVE	9	0
NEGATIVE	25	17

RTPCR	PERCENTAGE
SENSITIVITY	26.47%
SPECIFICITY	100%
POSITIVE PREDICTIVE VALUE	100%
NEGATIVE PREDICTIVE VALUE	40.47%

There were no False positive results with RTPCR making the Specificity and Positive predictive value as 100%. The Sensitivity was 26.47%(much higher than BACTEC) and Negative predictive value was 40.47%.

SENSITIVITY AND SPECIFICITY

HISTOPATHOLOGICAL EXAMINATION

HPE	MALIGNANT CASES	NON- MALIGNANT CASES
POSITIVE	12	0
NEGATIVE	5	34

HISTOPATHOLOGICAL EXAMINATION	PERCENTAGE
SENSITIVITY	70.6%
SPECIFICITY	100%
POSITIVE PREDICTIVE VALUE	100%
NEGATIVE PREDICTIVE VALUE	87.17%

There were no False positive results with Histopathological Examination making the Specificity and Positive predictive value as 100%. The Sensitivity was 70.6% and Negative predictive value was 87.17%.

DISCUSSION

Needle biopsy of the pleura can establish the diagnosis of a malignant pleural effusion. The percentage of positive pleural biopsies in patients with malignant pleural disease ranges from 39% to 75%. In general, pleural fluid cytology is superior to pleural biopsy in establishing the diagnosis of pleural malignancy. The diagnostic yield of Pleural biopsy in our study was 70% in Malignant cases.

In one study of 248 patients with tuberculous pleuritis who underwent needle biopsy of the pleura, the biopsy showed granulomas in 198 patients (80%), the acid-fast stain of the biopsy was positive in 64 (25.8%), the culture of the biopsy tissue was positive in 140 (56%), and at least one of the preceding three tests was positive in 227 (91%) (28). Polymerase Chain Reaction has also been tried on pleural biopsy specimens but it is not clear that the Polymerase Chain Reaction adds to the regular microscopic examination (29). In our study Histopathological examination diagnosed 67% of Tuberculous effusion . Reverse Transcriptase Polymerase Chain Reaction diagnosed 26.1% of Tuberculous effusion. BACTEC diagnosed 11.8% of Tuberculous effusion . 82.3% of Tuberculous cases were diagnosed by combining all three tests .

As there were no false positive results with any of the three tests (Histopathological examination, Reverse Transcriptase Polymerase Chain Reaction and BACTEC culture), they are highly Specific in diagnosing both Tuberculosis and malignancy. Both the Specificity and Positive predictive value were 100% with these tests. As these tests are highly specific we can start treatment even when any of these test is positive .

CLINICAL IMPLICATION

- Closed Pleural Biopsy can be used as a easy, quick, cost effective and relatively safe method to diagnose an exudative effusion not diagnosed by pleural fluid analysis.

- No complications occurred during the procedure except for a single case of Vasovagal shock (recovered).

- No post-procedure complications (such as Pneumothorax, hemorrhage or Infections of the pleural space).

- Results of Reverse Transcriptase Polymerase Chain Reaction are available within 24 hours and treatment can be initiated readily .

- BACTEC culture detects both Mycobacterium Tuberculosis and Non Tuberculous Mycobacterium .

- Resistance pattern of Mycobacterium Tuberculosis can be assessed by both BACTEC culture and Genetic analysis of the DNA detected using Reverse Transcriptase Polymerase Chain Reaction .

- Reverse Transcriptase Polymerase Chain Reaction targeting 85 B gene is very specific for Mycobacterium tuberculosis , so that it is negative for Non-Tuberculous Mycobacterium.

- m-RNA detected by Reverse Transcriptase Polymerase Chain Reaction has a half life for only few minutes and can detect only viable Mycobacterium .

CONCLUSION

- Combination of Histopathological examination, BACTEC culture and Reverse Transcriptase Polymerase Chain Reaction in closed Pleural biopsy has a greater diagnostic yield in diagnosing exudative effusions not diagnosed by Pleural fluid analysis .
- Histopathological examination diagnosed 67% of Tuberculous effusion .
- Reverse Transcriptase Polymerase Chain Reaction diagnosed 26.1% of Tuberculous effusion .
- BACTEC diagnosed 11.8% of Tuberculous effusion .
- Histopathological examination diagnosed 70% of Malignant effusion .
- 82.3% of Tuberculous cases were diagnosed by combining all three tests .
- 82.3% of Tuberculous cases were diagnosed by combining Histopathological examination and Reverse Transcriptase Polymerase Chain Reaction .

- Pleural biopsy diagnosed 79% of undiagnosed effusion .
- Closed Pleural Biopsy can be used as a easy, quick, cost effective and relatively safe method to diagnose an exudative effusion not diagnosed by pleural fluid analysis.

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